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Insecticidal activity of petroleum ether extract and essential oil of *Chenopodium ambrosioides* L. (Chenopodiaceae) against *Anopheles gambiae* (Diptera: Culicidae)

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Abstract: Tests were carried out to evaluate the toxicity of *Chenopodium ambrosioides* (Chenopodiacea) leaf against the 0 – 2 d old adult, 1st, 2nd, 3rd and 4th instar larvae of *Anopheles gambiae* (Diptera: Culicidae) as petroleum ether extract and essential oil respectively at ambient laboratory temperature. Thirty larvae at each instar stage were exposed to 50, 100, 250, 500, 750 and 1 000 mg/L of extract and essential oil in water, and the number of dead larvae recorded at 24 and 48 h post-exposure. Thirty adults were exposed to *C. ambrosioides* oil vapour in air-tight rectangular glass cages at 0.8, 1.6 and 2.4 μL/L and mortality recorded 24 h after exposure. The results showed that the test extract and essential oil were toxic to all larval stages and adults of *An. gambiae*. The median lethal concentration after 48 h exposure (48 h-LC_{50}) values show that the extract was most toxic to the 1st instar larvae (14.89 mg/L) followed by the 4th instar (18.90 mg/L) and least toxic to the 3rd instar (183.77 mg/L). The 48 h LC_{50} values showed that the essential oil was most toxic to the 4th (36.62 mg/L) followed by the 1st instar (90.75 mg/L) larvae. The computed LC_{50} value of *C. ambrosioides* oil against adult *An. gambiae* was 1.01 μL/L. The study highlights the potential of *C. ambrosioides* for the control *An. gambiae*.

Key words: Malaria; *Anopheles gambiae*; *Chenopodium ambrosioides*; insecticidal activity; bioassay; median lethal concentrations; essential oil; petroleum ether extract

1 INTRODUCTION

The use of plants as insect control agents is an agelong practice in Africa (Belmain and Stevenson, 2001; Ewete *et al.*, 2007), and *Chenopodium ambrosioides* (Chenopodiaceae) is a high ranking choice. In Malawi, Southern Africa; Congo, Central Africa and Benin Republic, west Africa, leaves of the shrub are dried and mixed with grains as protectant against insect infestation during storage (Delobel and Malonga, 1987; Malik and Naqvi, 1988; Tapondjou *et al.*, 2002). In Badagry Local Government Area, Lagos State, Nigeria, *C. ambrosioides* leaves are hung on the door posts as mosquito repellents by villagers. These practices are only observed in some cases as a mere allegiance to tradition and not because of proven or outstanding insecticdal or repellent efficacy (Delobel and Malonga, 1987). It has therefore become necessary to investigate the bioactivity of the shrub against various insect groups and non-target species with a view to maximally exploiting the potentials of the plant for insect control purposes. This is especially against the backdrop of recent elucidation of the constituents of this plant by Kasali *et al.* (2006).

The test insect species in this study, *Anopheles gambiae* (Diptera: Culicidae), is the vector of malaria (Amajoh *et al.*, 2002). In Ibeju-Lekki also in Lagos State, Nigeria, 83.1% of mosquito catches were *Anopheles* sp. (Amajoh *et al.*, 2002). Malaria infects 300 – 500 million people worldwide and accounts for over one million deaths annually (World Health Organization, 1996). Notably, 90% of the malaria infection occurs in Africa and at least one million African children die of the disease annually (Reeves, 1994; Coetzee, 1997; Egwunyega *et al.*, 1998; Tanner and Vlassoff, 1998; Taklehaimanot *et al.*, 2001). In Nigeria, malaria is an obstacle to deveopment because transmission rate is high as are super-infection (Denloye *et al.*, 2003). Tackling the disease is there highly considerable and one means of achieving this is source reduction, of which the rendering of breeding sites unsuitable for larval

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development is an important component. Synthetic insecticides have been used to control mosquitoes over time, but this leeds to adverse repercussions such as poisoning, environmental destruction and development of resistance in target species (Don-Pedro, 1980; Boroffice and Boroffice, 1993). This has encouraged exploration of various means including plant products as novel strategies that are cheap, locally available, easily applicatable and environmentally safe for the control of insect pests. Empirical determination of the toxicity of *C. ambrosioides* extracts and essential oil may therefore yield ideas for more effective use of the plant and its constituents for reducing the burden of malaria.

2 MATERIALS AND METHODS

2.1 Source and preparation of test plant species

Leaves of a portion of *C. ambrosioides* from Ipara in Badagry Local Government Area, Lagos State, Nigeria already analyzed by Kasali *et al.* (2006) for its chemical constituents was used as petroleum ether extract or essential oil.

2.2 Plant extract

2.2.1 Preparation of extract: Leaves used for the preparation of crude petroleum ether extract were first air-dried in the laboratory for 3 d at ambient temperature and relative humidity, and further dried for another 72 h in a wooden cabinet (1.0 m × 0.5 m × 1.0 m) fitted with 100 watts bulb, that provided about 42℃ temperature before pulverization into powder in a Binatone® blender (model no BLG 400). The powder was passed through sieve of 0.1 mm mesh size to standard particles. The leaf powder (500 g) was soaked in 1 L of petroleum ether for 48 h and filtered through Whatmann No. 1 filter paper (15 cm diameter). The filtrate was stored in labeled Kilner® jar while the residue was re-extracted for another 48 h after which it was filtered and the two filtrates pooled together. The combined filtrates were then dried over water bath at 50℃. The residue served as the petroleum ether leaf extract of *C. ambrosioides*. Graded concentrations of 50, 100, 250, 500, 750 and 1 000 mg/L of the crude extract were prepared by serial dilution using petroleum ether.

2.2.2 Test essential oil: Leaves of *C. ambrosioides* (500 g) already air-dried for 72 h on open laboratory bench were hydrodistilled in Clavenger apparatus for 6 h at 100℃ (Kasali *et al.*, 2006). The essential oil (1.0 mL) was mixed with 0.3 mL of hexane to make it soluble in water. Graded concentrations of 50, 100, 250, 500, 750 and 1 000 mg/L of the essential oil were prepared by serial dilution.

2.2.3 Experimental mosquitoes: *Anopheles gambiae* mosquito larvae and adults were obtained from cultures maintained in the Applied Entomology Research Laboratory, Lagos State University as previously described by Denloye *et al.* (2003).

2.3 Acute toxicity of *C. ambrosioides* against *An. gambiae* larvae

2.3.1 Petroleum ether extract: Thirty *An. gambiae* larvae in their 1st, 2nd, 3rd and 4th instars were transferred into 200 mL glass jars containing 10 mL of de-ionized water. From each of the graded concentrations of petroleum ether extract 3 mL was added to 90 mL of de-ionized water respectively, thoroughly mixed for 2 min and then transferred into exposure cups (200 mL transparent plastic cups). Controls consisted of 100 mL of de-ionized water. Each experiment and control was replicated four times. The larvae were then transferred into exposure cups containing either test *C. ambrosioides* extract or water, and maintained at 28 ± 1℃ for 24 or 48 h after treatment before survival was assessed. After exposure, larvae were washed with de-ionized water and transferred into recovery cups. A recovery time of 5 min was allowed, during which the water was agitated with a sterile stirrer to induce sinking of larvae to the bottom of the cups. Larvae that failed to swim to the water surface within 5 min were regarded as dead. Data obtained were used to compute percent mortality and median lethal concentration values.

2.3.2 Essential oil: A similar experiment was carried out as described above using essential oil instead of the petroleum ether extract of *C. ambrosioides* against *An. gambiae* larvae. The graded concentrations were used for bioassay to determine the acute toxicity of the essential oil against test larvae. Thirty 1st, 2nd, 3rd and 4th instar larvae were respectively exposed to each graded concentration in 200 mL plastic cups. A control was set up in each case exposing the same number of each larval stage to only deionized water. All experiments and controls were replicated four times. Survival was assessed, as previously described, every 24 h for 2 d. Mortality data generated were used to compute LC_{50} values to determine the toxicity of essential oil to the larvae.

2.4 Acute toxicity of dry film of *C. ambrosioides* petroleum ether extract to *An. gambiae* adult

Three aluminum-framed glass cages (0.5 m × 0.5 m × 0.5 m) which served as the exposure chambers were smeared with 1 mL of 50, 100 mg/L or water (control) and allowed to air-dry for 20 min. Thirty 0 – 2 d old adult *An. gambiae* were transferred into each cage and left for 24 h. Each treatment and control was replicated four times. After 24 h exposure, mosquitoes in each cage were assessed for mortality. Motionless mosquitoes unable to respond to gentle stroking of their legs with a fine brush bristle were counted dead. Mortality data obtained from this experiment were subjected to Analysis of Variance (ANOVA) and means separated by Least Significant Difference (LSD) at $P \leqslant 0.05$.

2.5 Toxicity of *C. ambrosioides* essential oil vapour against adult *An. gambiae*

Twenty 0 – 2 d old adult *An. gambiae* mosquitoes were transferred into four aluminum-framed glass cages (0.5 m ×0.5 m× 0.5 m) which served as the exposure chamber. All sides of the exposure chamber were sealed with transparent sticky tape to prevent vapour escape. Whatmann No. 1 filter paper (15 cm diameter) with attached white thread was treated with different volumes of the *C. ambrosioides* oil and suspended immediately in each exposure chamber at treatment rates of 0.8, 1.6 and 2.4 μL/L, respectively. A control was also set up using only dry filter paper. All treatments and control were replicated four times each. Survival was assessed after 24 h. Insects that were unable to move any part of the body after gentle stroking of their leg with fine brush bristle were counted as dead. Data collected were used to determine percent mortality and values of the LC_{50} of oil against adult *An. gambiae*.

2.6 Data analyses

The number of dead mosquito larvae or adults at all treatments in acute toxicity experiments were subjected to probit analysis following Finney (1971) with the aid of a computer software adapted by Don-Pedro 1996. Mean values of adult mosquito mortality in dry film experiment were compared by ANOVA and significant differences between them determined with Least Significant Difference statistic ($P \leqslant 0.05$) using Statistical Package for Social Scientists (SPSS) computer software (version 12.0). Data were square root transformed before ANOVA.

3 RESULTS

3.1 Toxicity of petroleum ether extract and essential oil of *C. ambrosioides* to larvae

The results showed that the test extract was toxic to all larval stages and adults of *An. gambiae*. The observed toxicity was increased as concentration of extract increased and also as the duration of exposure increased for the 48 h experimental time. The computed 24 h-LC_{50} values (Table 1) showed that the extract was most toxic to the 1st instar (61.94 mg/L) than any other larval stages while it was least toxic to the 3rd instar larvae (372.33 mg/L). Comparison of the 24 h and 48 h LC_{50} values showed that there was a significant increase in the toxicty of the test extract against *An. gambiae* as the values significantly decreased against each larval instar as evidenced by no overlap in the 95% confidence limits of the LC_{50} values (Table 1). The computed 48 h-LC_{50} values showed that the extract was more toxic to the 1st instar larvae (14.89 mg/L) followed by the 4th instar (18.90 mg/L) and least toxic to the 3rd instar (183.74 mg/L).

Table 1 Toxicity of *Chenopodium ambrosioides* extract to *Anopheles gambiae*

Treatment	*An. gambiae*	Time (h)	LC_{50} (mg/L)	Confidence limits	Slope ± *SE*	*df*	Probit line
Extract	1st instar	24	61.94	41.82 - 91.13	1.166 ± 0.02	4	$y = -2.09 + 11.66x$
	2nd instar		123.62	76.72 - 197.78	0.69 ± 0.02	4	$y = -1.45 + 0.69x$
	3rd instar		372.33	286.91 - 372.33	1.06 ± 0.01	4	$y = -2.71 + 1.06x$
	4th instar		129.93	105.04 - 160.52	1.58 ± 0.02	4	$y = -3.34 + 1.58x$
Extract	1st instar	48	14.89	4.83 - 43.34	0.76 ± 0.02	4	$y = -0.88 + 0.76x$
	2nd instar		41.05	21.16 - 78.56	0.83 ± 0.02	4	$y = -1.33 + 0.83x$
	3rd instar		183.74	135.98 - 25.81	0.96 ± 0.01	4	$y = -2.16 + 0.96x$
	4th instar		18.90	8.68 - 40.45	1.04 ± 0.03	4	$y = -1.33 + 1.04x$
Essential oil	1st instar	24	105.40	75.99 - 145.72	1.09 ± 0.02	4	$y = -2.203 + 1.09x$
	2nd instar		437.59	293.68 - 554.21	0.71 ± 0.01	4	$y = -1.87 + 0.07x$
	3rd instar		596.79	434.09 - 822.71	1.00 ± 0.02	4	$y = -2.77 + 1.00x$
	4th instar		248.68	210.78 - 293.31	1.18 ± 0.02	4	$y = -4.29 + 1.79x$
Essentialoil	1st instar	48	90.75	60.71 - 134.92	0.95 ± 0.01	4	$y = -1.85 + 10.95x$
	2nd instar		135.24	95.97 - 189.93	0.93 ± 0.01	4	$y = -1.99 + 0.93x$
	3rd instar		141.71	103.30 - 193.91	1.00 ± 0.02	4	$y = -2.14 + 1.00x$
	4th instar		36.62	23.97 - 55.53	1.45 ± 0.03	4	$y = -1.27 + 1.45x$
Oil vapour	Adult	24	1.01*	0.52 - 1.73	0.87 ± 0.03	1	$y = -0.22 + 0.87x$

* The unit of LC_{50} of oil vapour is μl/L.

The essential oil was more toxic after 48 h exposure to larvae exposed to water treated with essential oil of *C. ambrosioides* than after 24 h in this study (Table 1). The 48 h LC_{50} values showed that the essential oil was most toxic to the 4th instar larvae (36.62 mg/L) followed by the 1st instar larvae (90.75 mg/L) (Table 1).

3.2 Toxicity of dry film of *C. ambrosioides* petroleum ether extract to adult *An. gambiae*

The dry film of petroleum ether extract of *C. ambrosioides* was toxic to the adults of *An. gambiae*. Comparaison of mortality of adult *An. gambiae* at the treatments and control showed that the mortality of adult mosquitoes was significantly higher ($P > 0.05$) in the treatments than control (Fig. 1).

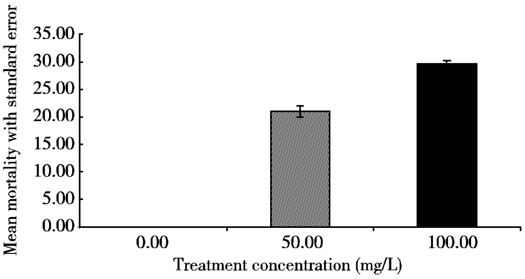

Fig. 1 Mortality of *Anopheles gambiae* adults exposed to dry film of *Chenopodium ambrosioides* petroleum ether extract

3.3 Vapour toxicity of *C. ambrosioides* to adult *An. gambiae*

The test oil caused mortality of adult *An. gambiae* at all concentrations after 24 h of exposure. The computed 24 h-LC_{50} value of the oil against the adults is 1.01 μL/L.

4 DISCUSSION

The toxicity of the petroleum ether extract and essential oil of *C. ambrosioides* against the larvae and adults of *An. gambiae* have been demonstrated in this study. The results of this study aptly justify the traditional uses of the plant by villagers in Badagry LGA of Lagos State, Nigeria who hangs the leaf on their door post and derives satisfaction that it reduces bites by mosquitoes (including *An. gambiae*) and the incidence of malaria in their communities. This is a departure from the traditional uses of plants in some African communities. Delobel and Malonga (1987) evaluated the insecticidal uses of certain plants including *C. ambrosioides* in Malawi and concluded that the use of some of these plants was unjustifiable since they did not demonstrate significant insecticidal properties and so were only being used as a token of allegiance to tradition rather than for proven efficacy. Against the backdrop of the fact that *An. gambiae* is the most efficient, most widespread and most difficult malaria vector to control (World Health Organization, 2003), the need to encourage the traditional uses of *C. ambrosioides* to control the insect becomes imperative.

In this regard it should be worthwhile to consider exploiting the plant material used in this study for incopration into insecticide treated bednets. One of the goals of the widely accliamed Roll Back Malaria (RBM) summit held in Abuja, Nigeria in April 2000 is a 50% reduction in Malaria burden in Africa by 2010. One of the intervention strategies is identified at the summit is the use of insecticide treated nets (ITNs) for which it was targetted that 60% of those at risk for malaria especially pregnant women and children ≤ 5 years old will benefit from a suitable combination of personal and community protective measurs such as ITNs. Since *C. ambrosioides* is locally available in African communities, and with its mosquitocidal effect demostrated in this study, there is a need to further exploit the plant for treating nets in the local communities.

One of the problems encountered in the ethnobotanical uses of plants is the lack of knowledge of their chemical constituents. This problem has been partly solved in the case of *C. ambrosioides*. Analysis of a portion of the plant used in this study by Kasali *et al.* (2006) showed that its constituents are sabinene, β-pinene, α-terpenene, p-cymene, limonene, (*E*)-β-ocimene, γ-terpinene, 1,4-epoxy-p-menth-2-ene, 1,2,3,4-diepoxy-p-menthane and phytol. Some of these constituents of essential oils of some plants such as *Citrus* species, which peels are dried and burnt to repel mosquitoes in Nigeria (Don-Pedro, 1985), and have been shown to be toxic to some other insects (Don-Pedro, 1996). An understanding of the mosquitocidal activity of these constituents should go a long way to enhance their further exploitation for mosquito and malaria control.

The results of this study provides a scientific rationale for the local uses of *C. ambrosioides* against mosquitoes in Badagry LGA, Lagos State, Nigeria and should serve as a basis to introduce the plant and

its uses in this regard to other communities where it is not presently being used for the same purpose.

References

Amajoh CN, Oduoko JB, Mosanya ME, 2002. Preliminary investigations on malaria in the Atlantic coastal margin of Ibeju-Lekki, Lagos State, Nigeria. *Journal of Malaria in Africa and the Tropics*, 1 (1): 17 - 19.

Belmain S, Stevenson P, 2001. Ethnobotanicals in Ghana: Reviving and modernizing age-old farmer practice. *Pesticide Outlook*, 10: 233 - 238.

Boroffice OB, Boroffice RA, 1993. Pesticide usage in Nigeria and the health implication. *Nigerian Journal of Research and Review in Science*, 1: 115 - 117.

Coetzee M, 1997. Malaria in Africa. In: Proceedings of the Joint Congress of the Entomological Society of Southern Africa and the African Association of Insect Scientists, 30 June - 4 July 1997, Stellenbosch, South Africa.

Delobel A, Malonga, 1987. Insecticidal properties of six plant materials against *Caryedon serratus* (OL). *Journal of Stored Product Research*, 23 (3): 173 - 176.

Denloye AA, Makanjuola WA, Babalola OO, 2003. Toxicity and repellent effects of crude aqueous extracts of garlic (*Allium sativum*) on larval and adult *Anopheles mosquitoes*. *African Entomology*, 11 (2): 287 - 290.

Don-Pedro KN, 1980. A population explosion of *Aphis crassivora* Koch following DDT application in a cowpea plot (*Vigna unguiculata*) in Nigeria. *Journal of Natural History*, 14: 617 - 619.

Don-Pedro KN, 1985. Toxicity of some citrus peels to *Dermestes maculatus* Deg. and *Callosobruchus maculatus* (F). *Journal of Stored Product Research*, 21(1): 31 - 34.

Don-Pedro KN, 1996. Investigation of single and joint fumigant insecticidal action of citruspeel oil components. *Pesticide Science*, 46: 79 - 84.

Egwunyega OA, Ajayi JA, Dublinska-Popov DD, 1998. Transplacental passage of *Palsmodium falciparum* and seroevaluation of newborn in northern Nigeria. *Journal of Communicable Diseases*, 27 (2): 77 - 83.

Ewete FK, Ashimolowo OR, Adohi EA, 2007. Survey of plants used in maize storage in Abeoukuta north local government area and efficacy of selected species against *Sitophilus zeamais* Mostchulsky (Coleoptera; Curculionidae). *Nigerian Journal of Entomology*, 24: 119 - 126.

Finney DJ, 1971. Probit Analysis. 3rd ed. Cambridge University Press, London.

Kasali AA, Ekundayo O, Paul C, Konig WA, Eshilokun AO, Ige B, 2006. 1, 2: 3, 4-Diepoxy-p-menthane and 1, 4-epoxy-p-menth-2-ene: Rare monoterpenoids from the essential oil of *Chenopodium ambrosioides* L. var *ambrosioides* leaves. *Journal of Essential oil Research*, 18 (1): 13 - 15.

Malik MA, Naqvi SHM, 1988. Screening of some indigenous plants as repellents or antifeedants for stored grain insects. *Journal of Stored Product Research*, 20 (1): 41 - 44.

Reeves PA, 1994. Malaria treatment in Vanauty: New national treatment guidelines. *Papua New Guinea Medical Journal*, 37 (3): 181 - 188.

Taklehaimanot A, Keusch G, Binder S, 2001. Malaria. *Emerging Infectious Diseases*, 7 (3): 546 - 547.

Tanner M, Vlassoff C, 1998. Treatment-seeking behaviour for malaria: a typology based on endemicity and gender. *Social Science and Medicine*, 46 (4 - 5): 523 - 532.

Tapondjou LA, Adler C, Bouda H, Fontem DA, 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of Stored Product Research*, 38: 395 - 402.

World Health Organization, 1996. Malaria and insecticide treated nets. *Tropical Disease Research*, 50: 4 - 5.

World Health Organization, 2003. Africa Malaria Report. World Health Organization, Geneva.

土荆芥的石油醚提取物及精油对冈比亚按蚊的杀虫活性

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摘要：在室内条件下测试了土荆芥 *Chenopodium ambrosioides* 叶片的石油醚提取物及精油对冈比亚按蚊 *Anopheles gambiae* 0～2 d龄成虫和1～4龄幼虫的毒性。每一龄期各取30头幼虫分别接触50,100,250,500,750和1 000 mg/L提取物和精油,于24 h和48 h后记录死亡幼虫的数目。30头成虫在密封的长方形玻璃笼子中分别接触0.8,1.6,2.4 μL/L土荆芥精油蒸气, 24 h后记录死亡率。结果表明,测试的提取物和精油对冈比亚按蚊的各龄幼虫和成虫均具有毒性。48 h致死中浓度(LC_{50})的测试结果表明,石油醚提取物对1龄幼虫的毒性最强(14.89 mg/L),其次是对4龄幼虫(18.90 mg/L),对3龄幼虫的毒性最低(183.77 mg/L); 精油对4龄幼虫毒性最强(36.62 mg/L),其次是对1龄幼虫(90.75 mg/L)。推算的土荆芥精油对冈比亚按蚊的LC_{50}为1.01 μL/L。本研究揭示了土荆芥对冈比亚按蚊的防治潜力。

关键词：疟蚊; 冈比亚按蚊; 土荆芥; 杀虫活性; 生测; 致死中浓度; 精油; 石油醚提取物

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